3881-Pos Board B609
The Structural Dynamics of Alpha-Tropomyosin on F-Actin Shape the Overlap Complex between Adjacent Tropomyosin Molecules
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Coiled-coil tropomyosin, localized on actin filaments in virtually all eukaryotic cells, serves as a gatekeeper regulating access of the motor protein myosin and other actin-binding proteins onto the thin filament surface. In order to form a continuous cable on thin filaments that is free of gaps, adjacent tropomyosin molecules polymerize head-to-tail by means of a short (~9-10 residue) overlap. Thus to characterize the overlap structure, several laboratories have engineered peptides to mimic the N/C-terminal tropomyosin association. The overlapping domains formed show a compact N-terminal coiled-coil inserting into a partially opened C-terminal partner, where the opposing coiled-coils at the overlap junction face each other at up to ~90° twist angles. Here, Molecular Dynamics simulations were carried out to determine constraints on the formation of the tropomyosin overlap complex and to assess the amount of twisting exhibited by full-length tropomyosin when bound to actin. With the exception of the last 20 to 40 C- and N-terminal residues, we find that the average tropomyosin structure closely resembles a "canonical" model proposed in the classic work of McLachlan and Stewart, displaying perfectly symmetrical supercoil geometry matching theF-actin helix with an integral number of coiled-coil turns, a coiled-coil helical pitch of 137 Å, a superhelical pitch of 770 Å, and no localized pseudo-rotation. Over the middle 70% of tropomyosin, the average twisting of the coiled-coil deviates only by 10° from the canonical model and the torsional freedom is very small (std. dev. of 7°). This small degree of twisting cannot yield the orthogonal N- and C-termini configuration observed experimentally. However, in marked contrast, considerable coiled-coil unfolding, splaying and twisting at N- and C-terminal ends is observed, providing the conformational plasticity needed for head-to-tail nexus formation.

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Myosin Binding to Human Cardiac Thin Filaments Containing Tropomyosin Carrying DCM & HCM Mutations; Fitting of Complex Binding Transients
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Myosin S1 binds to pyrene labelled thin filaments in a calcium dependent manner. When mixing an excess of S1 with thin filaments at low calcium the transient is complex in form and requires a complete model of the regulated binding to fit the data. Using the recently published Monte Carlo version of the Mckillop & Geeves model (REF) we have now fitted a complete set of calcium dependent myosin binding transients to thin filaments containing WT human tropomyosin with human cardiac troponin. In addition we have analysed the data for 5 tropomyosin mutations carrying HCM (E175N, & E180G) or DCM mutations (E54K , E40K and D230N) in one or both Tm chains of the dimer. All transients can be well described by the model and for each case the calcium dependent data can be described by a single set of parameters with a change only in the apparent value and calcium dependence of K_B (the equilibrium constant between the blocked and closed states of the filament) small secondary effects may be present in value of K_T (equilibrium between closed and open states). For the HCM mutations the value of K_B showed enhanced calcium sensitivity with little change in the value of K_B at high and low calcium. In contrast, preliminary data for DCM mutations showed little change in the calcium dependence of K_B, but larger changes in the low calcium value of K_B. This could mean that the DCM mutations turn off the thin filament more effectively. All other constants remain unchanged within experimental error.
Supported by the Welcome Trust, NIH R01 AR048776 and Serbian Ministry of Science grants III41007 and OI174028.

3883-Pos Board B611
Mg^{2+} Dependent Modulation of Striated Muscle Myosin ATPase by Thin Filament Components
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While it's been reported that non-muscle myosin such as myosin V can be regulated by Mg^{2+}, striated muscle myosin modulation by Mg^{2+}-concentration dependency, especially in relation to thin filament components, has not been well studied up to now. It has been suggested that ADP release (product release) from myosin V is affected by Mg^{2+} concentration. Therefore, Mg^{2+}-dependent modulation of striated muscle myosin ATPase was explored in the presence of reconstituted muscle thin filament components, i.e. actin, tropomyosin and troponin. ATPase activity assays and Nuclear Magnetic Resonance (NMR) spectroscopy were employed to understand the mechanisms of ATP hydrolysis by striated muscle myosin and the role of Mg^{2+} in the enzymatic activity. An NMR technique called Water Ligand-Observed Gradient Spectroscopy (WaterLOGSY) was utilized to probe if the interaction of myosin with ADP (one of the products resulted from ATP hydrolysis) is different depending on Mg^{2+} concentration as well as the presence of muscle thin filament components. Our WaterLOGSY data demonstrated that this technique is a promising method to monitor ADP binding to myosin head S1 even in the presence of actin filaments and that there was a change in the appearance of peaks between with and without tropomyosin at a low Mg^{2+} concentration. This set of results indicates that the way by which myosin binds ADP is different depending on the states of thin filaments. Moreover, actomyosin S1 ATPase rate decreased as Mg^{2+} concentration increased. When tropomyosin was reconstituted in the thin filaments, the ATPase rate decreased more rapidly compared with actomyosin alone. Thus we conclude that Mg^{2+} plays an important role in striated muscle myosin ATPase and that regulatory components such as tropomyosin modulate Mg^{2+} dependency of myosin ATPase in the striated muscle.

3884-Pos Board B612
Kinetic and Structural Characterization of Calcium Sensitizer Action on Thin Filament Function using FRET
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The use of novel calcium sensitizing agents in the treatment of heart disease offers therapeutic value for patients suffering from a particularly prevalent and recalcitrant condition, however the elusive mechanisms of action for these drugs prevent increased and improved utilization of such agents clinically. Ideally the calcium sensitizer impact on the thin filament could be monitored directly in a physiologically relevant and dynamic way while still capturing the molecular level mechanisms involved. With that aim a homo-FRET scheme was developed which monitors the N-domain opening of the calcium binding subunit of cardiac troponin (N-cTnC) by labeling with TAMRA at cTnC(cys-13) and cTnC(cys-51). Using this novel FRET design the calcium binding properties of reconstituted troponin and their deactivation kinetics were measured in the presence of calcium sensitizers Levosimendan, Bepridil, Pimobendan, and EMD-57033 at various levels of *in vitro* reconstitution. We hypothesized that depending on the mechanism for each calcium sensitizer the effects on cTnC calcium binding and deactivation kinetics would vary based on the level of reconstitution. We expect that new insight into which thin filament proteins are necessary for sensitizer action will yield a clearer picture of the molecular level mechanisms underlying cardiotonic action. Although the study is currently ongoing preliminary results show significant sensitization for all four drugs in reconstituted ternary troponin compared to control and that this effect is abrogated in samples containing only cTnC and cardiac troponin inhibitory subunit (cTnI). Deactivation kinetics show a decreased transition rate for Levosimendan, Bepridil and Pimobendan but not for EMD-57033 both at the ternary troponin level and at the cTnI-cTnC level. Results from measurements with reconstituted thin filament preparation will be also discussed.

3885-Pos Board B613
Monitoring Cardiac Troponin Structural Changes using In-Situ Time-Resolved FRET: Implications on the Regulatory Roles of Cross-Bridges and Sarcomere Length
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During cardiac thin filament activation, the N-domain of Ca^{2+}-binding cardiac troponin C (N-cTnC) interacts with the actomyosin inhibitory troponin I (cTnI) subunit, which concomitantly opens the cTnC N-domain and leads to force generation. Recently, we used in situ steady state FRET measurements based on N-cTnC opening to determine that strongly bound cross-bridges (XBs) stabilize this Ca^{2+}-sensitizing N-cTnC-cTnI interaction. However, the method was unable to determine how N-cTnC opening is affected by